

# Spatial Analysis of Forage Grass Trials across Locations, Years, and Harvests

K. F. Smith and M. D. Casler\*

## ABSTRACT

Spatial analyses of yield trials are a powerful method of adjusting treatment means for spatial variation and improving statistical precision of mean estimation. Because yield trials are typically repeated across multiple locations and years, spatial analysis methods must be adapted for combined analyses across locations and years. The objective of this study was to evaluate the relative efficiency of nearest neighbor analysis (NNA) across locations and years for several perennial forage grass trials. Three spatial adjustment methods were developed: preadjustment based on total forage yield, postadjustment based on total forage yield, and preadjustment based on forage yield of individual harvests. For cool-season grasses on a multiple-harvest management, NNA had relative efficiencies of 105 to 135% across locations, years, and trials. Within trials, there was some consistency across harvests, resulting in greater improvements in precision for adjustment based on total yield. Across locations and years, the three spatial adjustment methods always ranked the same in relative efficiency: preadjustment by harvest > preadjustment of total yield > postadjustment of total yield. The advantage of the preadjustment methods was likely due to fitting heterogeneous slopes (adjustment factors) across locations, years, and/or harvests. In contrast, trials with a single-harvest management for biomass production always had relatively low relative efficiency of NNA. Trial operators should assess the relative efficiency of NNA on early harvests from all locations within a trial and if the relative efficiencies are large, they should consider the use of NNA across locations and years to adjust entry means.

PERENNIAL FORAGE GRASS SPECIES are routinely tested for improvements in forage yield through the use of replicated plot trials in a number of years and locations. These cultivar evaluation programs are essential for making choices between forage grass cultivars and also for assessing whether new cultivars are broadly suited across a range of environments or possess more specific adaptation to certain environmental niches.

The majority of forage cultivar evaluation trials are sown in a randomized complete block (RCB) design (APPEC, 1996). While the RCB design may be an effective way of controlling spatial variation in field trials in one direction, it is ineffective when the spatial variability is continuous in two directions, leading to considerable within-block variability (Lin et al., 1993). There has been a marked change in the way that multi-environment trial data from annual grain crop variety testing trials are analyzed with a move toward spatial analysis (Gleeson and Cullis, 1987; Cullis and Gleeson, 1989) to better accommodate the plot-to-plot variation observed in

field trials. The use of row-column analysis or neighbor analysis has been shown to increase the precision of a large number of grain yield trials (Cullis and Gleeson, 1989; Cullis and Gleeson, 1991; Kempton et al., 1994).

Recent analyses of forage grass cultivar trials of a range of cool season forage species have shown that it is possible to improve the precision of cultivar yield estimates within a location through both optimizing the number of replicates sown, based on the likely differences between the cultivars under test, and utilizing statistical analyses that account for spatial variability in plot yield (Casler, 1999a,b; Smith and Kearney, 2002). When RCB designs were compared with lattice designs and NNA in a comparison of 27 perennial cool-season grass trials, NNA was shown to provide more precise estimates of mean forage yield than either the lattice or RCB designs (Casler, 1999b). The improvements in precision of entry means were shown to be incremental with an average improvement in precision of 15% due to the use of RCB designs, an additional 17% due to the lattice analysis, and a further 22 to 30% due to trend analysis or NNA (Casler, 1999b).

These improvements in the precision of the estimation of cultivar herbage yield are of great importance given the rapid increase in the number of forage grass cultivars on the market, the relatively low rates of genetic gain for forage yield (0.1–0.5% yr<sup>-1</sup>; Van Wijk and Reheul, 1991; Casler, 1998; Casler et al., 2000), and the reduction in funds available for cultivar testing in a number of countries. Nearest neighbor adjustment of cultivar means for individual trials provides improved precision for cultivar means, but does not provide a direct assessment of cultivar × environment interactions, which require a combined analysis across locations or years. Supplemental analysis of adjusted cultivar means could provide this information (Cullis et al., 1998), but would not provide a test of each cultivar × environment component (location, year, and location × year). Thus, the need still exists to develop techniques to allow for analysis of spatially adjusted means across environments and years as forage cultivar trials are usually conducted across 2 to 3 yr in a number of locations (Casler, 1999a,b).

The objective of this study was to evaluate several methods to use NNA to account for spatial variability in the yields of forage plots from nine separate cultivar evaluation trials conducted across locations and years. The trials cover two distinct classes of forage cultivar evaluations: multiple-harvest hay trials of cool-season grasses, for which season-total forage yield is the trait of interest, and single-harvest biomass trials of a warm-season grass.

K.F. Smith, Agriculture Victoria, CRC for Molecular Plant Breeding, Pastoral and Veterinary Inst., Private Bag 105, Hamilton, VIC 3300, Australia; M.D. Casler, USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI 53706-1108. Received 19 Feb. 2003. \*Corresponding author (mdcasler@wisc.edu).

Published in Crop Sci. 44:56–62 (2004).  
© Crop Science Society of America  
677 S. Segoe Rd., Madison, WI 53711 USA

**Abbreviations:** LSR, least significant range; NNA, nearest neighbor analysis; Pre-IH, preadjustment by individual harvests; RCB, randomized complete block.

## MATERIALS AND METHODS

This study used data collected from nine experiments, representing four forage grass species (smooth brome grass, *Bromus inermis* Leyss.; orchardgrass, *Dactylis glomerata* L.; hybrid wheatgrass, *Elytrigia*  $\times$  *muctonata* (Opiz ex Bercht) Prokud; and switchgrass (*Panicum virgatum* L.). Trials, defined herein as one site of an experiment, were sown in a RCB design at up to four locations per experiment, 2 or 3 harvest years, and one to three forage yield harvests per year (Table 1). Plot sizes ranged from 1.5 to 4.5 m<sup>2</sup> for the cool-season grasses and from 2.4 to 6.9 m<sup>2</sup> for switchgrass. Each of the nine experiments contained a different set of entries. Trials were sown in 1992 (SB2), 1997 (SW1, OG2, and SB3), 1998 (SW2, OG1, SB1, and HW), or 1999 (SW3).

Forage yield was determined by harvesting each plot with a flail-type harvester at a cutting height of  $\approx 9$  cm. Dry matter determinations were made on random 300- to 500-g forage samples and were used to adjust plot yields to a dry-matter basis. Cool-season grass trials were harvested three times per year: early June (just after heading), early August, and late October. Switchgrass trials were harvested in late summer, just after anthesis. Cool-season grass trials generally received 56 kg N ha<sup>-1</sup> at the beginning of each harvest-growth period, while switchgrass received 100 kg N ha<sup>-1</sup> in early spring. Dry matter yields for each plot were summed across all harvests within each year to give the annual forage production for a given plot.

### Analyses within Locations and Years

For each trial, the annual forage yield and the forage yield at individual harvests within years were analyzed with a RCB design. The annual forage yield and the yield at individual harvests were also subjected to NNA with two covariates (Casler, 1999b). The two covariates were

$$R_{kl} = (e_{k,l-1} + e_{k,l+1})/2$$

and

$$C_{kl} = (e_{k-1,l} + e_{k+1,l})/2,$$

where  $R_{kl}$  and  $C_{kl}$  are the two covariates corresponding to the plot located in row  $k$  and column  $l$  of the trial grid and the four values of  $e_{k,l}$  are the residuals to the right, left, top, and bottom of the  $kl$ th plot. The variable  $R_{kl}$  is the mean of the residuals from plots in adjacent rows to the  $kl$ th plot and  $C_{kl}$  is the mean of the residuals from plots in adjacent columns to the  $kl$ th plot. Nearest neighbor analyses with two covariates ( $R$ ,  $C$ ), treatment of edge and corner plots, and program code for computing the nearest neighbor covariates were as described by Brownie et al. (1993). Mixed model code for SAS is described by Littell et al. (1996).

To develop a measure of experimental precision comparable with that obtained with the RCB analysis, the individual entry standard errors from NNA were squared and averaged across entries within each analysis to derive a pooled variance of adjusted entry means, equal to the square of the SAV value (square root of average variance) computed by Brownie et al. (1993). The relative efficiency of NNA was expressed as the ratio of the pooled variance of the entry means from RCB and NNA (Casler 1999b).

### Combined Analyses across Locations and Years

Raw data were analyzed by mixed models analysis within the Statistical Analysis System (Littell et al., 1996), using the RCB model without spatial analysis combined across locations and years. The linear model was:

$$Y_{ijkl} = M + L_l + \beta_{jl} + Y_k + LY_{kl} + \gamma_{jkl} + E_i + EL_{il} + \delta_{ijl} + EY_{ik} + ELY_{ikl} + \varepsilon_{ijkl},$$

where  $M$  = the grand mean,  $L_l$  = the fixed effect of locations,  $Y_k$  = the fixed effect of years,  $E_i$  = the fixed effect of entries, and the Greek letters all refer to random error terms. Locations were assumed to be fixed because they were not chosen at random from any well-defined target population. Years were assumed to be fixed because they were a measure of stand age. Inferences for both years (stand ages) and locations

**Table 1. Description of nine forage or biomass experiments repeated across multiple locations and years.†**

| Experiment (Species)     | Location          | y | h     | N  | Rep. | Rows | C  |
|--------------------------|-------------------|---|-------|----|------|------|----|
| OG1 (Orchardgrass)       | Arlington, WI     | 2 | 3     | 17 | 4    | 16   | 8  |
|                          | Ashland, WI       | 2 | 3     | 17 | 4    | 16   | 8  |
|                          | Marshfield, WI    | 2 | 3     | 17 | 4    | 16   | 8  |
| OG2 (Orchardgrass)       | Rock Springs, PA  | 2 | 3     | 30 | 4    | 24   | 5  |
|                          | Charlottetown, PE | 2 | 2,3   | 30 | 4    | 24   | 5  |
|                          | Ames, IA          | 2 | 3     | 30 | 4    | 24   | 5  |
|                          | Arlington, WI     | 2 | 2,3   | 30 | 4    | 24   | 5  |
|                          | Ashland, WI       | 2 | 2,3   | 10 | 16   | 32   | 8  |
| SB1 (Smooth brome grass) | Marshfield, WI    | 2 | 3     | 10 | 16   | 32   | 8  |
|                          | Ashland, WI       | 2 | 3     | 10 | 16   | 32   | 8  |
|                          | Marshfield, WI    | 2 | 3     | 10 | 16   | 32   | 8  |
| SB2 (Smooth brome grass) | Arlington, WI     | 2 | 3,2   | 32 | 3    | 12   | 8  |
|                          | Ashland, WI       | 2 | 3,2   | 32 | 3    | 12   | 8  |
|                          | Lancaster, WI     | 2 | 1,3   | 32 | 3    | 12   | 8  |
| SB3 (Smooth brome grass) | Arlington, WI     | 3 | 2     | 24 | 4    | 12   | 8  |
|                          | Ashland, WI       | 3 | 2,3,3 | 24 | 4    | 12   | 8  |
|                          | Marshfield, WI    | 3 | 2,3,2 | 24 | 4    | 12   | 8  |
|                          | Arlington, WI     | 2 | 2     | 10 | 16   | 32   | 8  |
| HW (Hybrid wheatgrass)   | Ashland, WI       | 2 | 3     | 10 | 16   | 32   | 8  |
|                          | Marshfield, WI    | 2 | 3     | 10 | 16   | 32   | 8  |
|                          | Brookings, SD     | 3 | 1     | 6  | 4    | 12   | 6  |
| SW1 (Switchgrass)        | Arlington, WI     | 3 | 1     | 6  | 5    | 12   | 6  |
|                          | Mead, NE          | 2 | 1     | 21 | 6    | 18   | 7  |
| SW2 (Switchgrass)        | Stillwater, OK    | 2 | 1     | 20 | 5    | 20   | 5  |
|                          | Arlington, WI     | 2 | 1     | 20 | 5    | 20   | 5  |
|                          | Spooner, WI       | 2 | 1     | 20 | 3    | 12   | 5  |
|                          | Arlington, WI     | 2 | 1     | 49 | 6    | 24   | 12 |
| SW3 (Switchgrass)        | Marshfield, WI    | 2 | 1     | 49 | 6    | 24   | 12 |

† y = number of years, h = number of harvests per year or number of harvests in each consecutive year, N = number of entries, Rep. = number of replicates, Rows = total number of rows, and C = total number of columns.

were limited to those used in each trial. Years were treated as a repeated measure with compound symmetric covariance structure (Littel et al., 1996). Replicates were assumed to be random and entries were assumed to be fixed.

Spatial analysis, combined across locations and years, was achieved by three different methods: preadjustment based on total forage yield (Pretotal), postadjustment based on total forage yield (Posttotal), and preadjustment by individual harvests (Pre-IH).

**Preadjustment based on total forage yield.** Plot yields from multiple harvests within a year (when present) were summed to give total annual forage yields. Total forage yields within each location and year were adjusted for spatial variation by an analysis with the two NNA covariates, excluding class variables (replicates and entries). The residuals from these analyses, which retained all information on entries, were restored to their original scale by addition of the grand mean. These values, spatially adjusted total forage yields within locations and years, were combined into a single data file and analyzed with the mixed model above without the two NNA covariates. Error df were reduced by  $2ly$  ( $l$  = number of locations,  $y$  = number of years) to account for the preadjustment fitting two NNA covariates for each location-year combination.

**Postadjustment based on total forage yield.** Plot yields from multiple harvests within a year (when present) were summed to give total annual forage yields. The NNA covariates were computed for total forage yield values within each location and year. The combined analysis was then performed on raw data, adjusting for spatial variation by use of the two NNA covariates added to the mixed model above.

**Preadjustment by individual harvests.** This method was as described for Pretotal, except when there were multiple harvests within a year. In these cases, individual-harvest forage yields were adjusted for spatial variation as described for Pretotal. Adjusted values for each harvest were rescaled by adding the grand mean, then summed within years, and analyzed by the mixed model above. Error df were reduced by the total number of NNA covariates fit, as described for Pretotal.

The results of each method were compared with those obtained by RCB analysis. The NNA adjustments to plot means in each trial were evaluated according to the relative efficiency of the adjustments as described for the analyses within locations and years. The ability to detect differences among entry means for each method of analysis was evaluated by the LSD0.05 and the LSD expressed as a percentage of the range of entry means within a trial (least significant range [LSR] of Casler and Undersander, 2000). The LSR  $[100(\text{LSD})/\text{Range}]$  expresses the LSD value as a percentage of the range among entry means, providing a relative measure of the extent to which entry differences can be detected. Spearman rank correlation coefficients were calculated between entry means computed from NNA and RCB analyses.

## RESULTS AND DISCUSSION

### Analyses within Locations and Years

The average relative efficiency of NNA for the 108 individual seasonal forage yield harvests of the hay cutting trials reported in this study was 121% (range 93 to 224) (Table 2). This value of 121% is comparable with the values of 122 to 130% reported for total annual forage yield in a different set of cool-season grass trials (Casler, 1999b) and was only slightly lower than 159% reported for two-dimensional NNA of cereal grain yield

trials (Kempton et al., 1994). When the yield of the plots was analyzed as the sum of all harvests within a year, the average relative efficiency of NNA compared with RCB analysis was 123%.

For the cool-season species with multiple harvests within each year, there was a significant ( $P < 0.001$ ) relationship between the relative efficiency of NNA of the annual forage yield and the average relative efficiency of the NNA of individual harvests within a year (Fig. 1). Furthermore, the slope of the regression of relative efficiency for total forage yield vs. weighted average relative efficiency across harvests was significantly  $>1$  ( $P < 0.001$ ). While the effects of spatial heterogeneity within a trial may have greater or lesser effects on the plot yields at individual harvests, there were consistent effects across harvests that were identified when the plot yields were expressed as annual totals. These effects appear to be partly summative (positively correlated across harvests), resulting in greater adjustments for total forage yield than for the weighted average across harvests, particularly for those trials with the greatest amount of spatial variation (Fig. 1). Such effects would include factors such as variation in soil profile that remain consistent throughout the trial but may have greater or lesser effects on plot yield due to other climatic factors such as moisture availability. For field locations with substantial spatial variation, covariate variables appear to more accurately reflect the true spatial distribution of forage yield potentials when computed from sums across multiple harvests than based on individual harvests.

In contrast to the data from the multiple-harvest trials, the average relative efficiency of NNA for switchgrass trials was only 104% of that obtained with RCB analyses (range 91 to 126) (Table 3). This may have been due to the fact that these data represent only three trials. However, given that they represent data from several different years and locations and that the relative efficiency of NNA was relatively constant across locations, years, and trials, it is possible that single-harvest biomass trials are less sensitive to spatial heterogeneity. The extreme photoperiod sensitivity of switchgrass (Benedict, 1941) may be partly responsible for the observed plot-to-plot uniformity relative to the cool-season grasses. The single-harvest management may also contribute to plot-to-plot homogeneity if there are buffering or compensatory growth effects that accumulate throughout the growing season.

### Analyses across Locations and Years

For the cool-season grass trials, NNA across locations and years had a relative efficiency between 105 and 135% compared with RCB (Table 4). The relative efficiency of NNA varied considerably among trials, with the two orchardgrass trials showing the highest efficiencies and hybrid wheatgrass trial the lowest efficiencies. All relative efficiency values were greater than 100%, indicating the potential value of NNA to describe spatial variability within blocks of the randomized block design,

**Table 2. Relative efficiency of nearest neighbor analysis compared with randomized complete block analysis for individual harvests and total forage yield within individual years and locations for six forage grass experiments.**

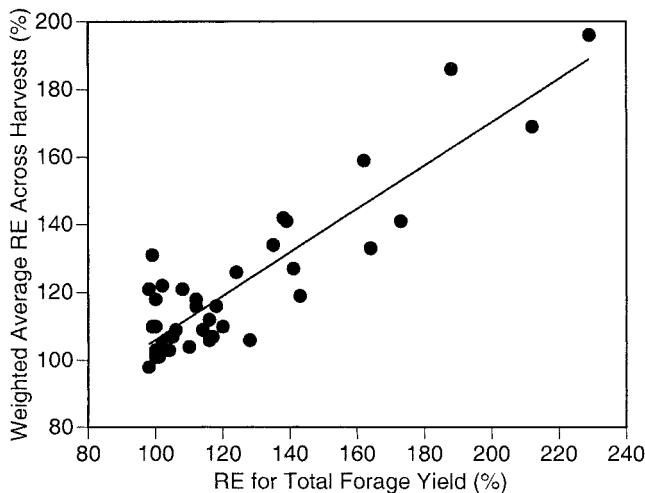
| Experiment†  | Location-Year   | Harvest 1   | Harvest 2 | Harvest 3 | Total yield |
|--------------|-----------------|-------------|-----------|-----------|-------------|
|              |                 | %           |           |           |             |
| OG1          | Arlington-1     | 115         | 127       | 109       | 116         |
|              | Arlington-2     | 114         | 107       | 119       | 116         |
|              | Ashland-1       | 110         | 132       | 100       | 111         |
|              | Ashland-2       | 127         | 114       | 122       | 108         |
|              | Marshfield-1    | 107         | 110       | 183       | 102         |
| OG2          | Marshfield-2    | 100         | 105       | 106       | 100         |
|              | Ames-1          | 142         | 132       | 106       | 163         |
|              | Ames-2          | 147         | 178       | 158       | 161         |
|              | Rock Springs-1  | 93          | 117       | 124       | 100         |
|              | Rock Springs-2  | 139         | 100       | 94        | 111         |
|              | Charlottetown-1 | 128         | 105       | —‡        | 111         |
|              | Charlottetown-2 | 119         | 111       | 224       | 137         |
|              | Arlington-1     | 115         | 99        | 105       | 114         |
|              | Arlington-2     | 113         | 126       | —         | 143         |
|              | SB1             | Arlington-1 | 138       | 117       | —           |
| Arlington-2  |                 | 109         | 106       | 102       | 106         |
| Ashland-1    |                 | 137         | 100       | 105       | 138         |
| Ashland-2    |                 | 109         | 103       | 109       | 102         |
| Marshfield-1 |                 | 120         | 108       | 113       | 118         |
| Marshfield-2 |                 | 102         | 109       | 100       | 109         |
| SB2          |                 | Arlington-1 | 97        | 116       | 128         |
|              | Arlington-2     | 191         | 124       | —         | 213         |
|              | Ashland-1       | 97          | 143       | 127       | 97          |
|              | Ashland-2       | 183         | 163       | —         | 173         |
|              | Lancaster-1     | 185         | —         | —         | 185         |
|              | Lancaster-2     | 153         | 98        | 127       | 99          |
|              | SB3             | Arlington-1 | 107       | 98        | —           |
| Arlington-2  |                 | 115         | 186       | —         | 141         |
| Arlington-3  |                 | 212         | 123       | —         | 229         |
| Ashland-1    |                 | 114         | 98        | —         | 101         |
| Ashland-2    |                 | 112         | 153       | 110       | 97          |
| Ashland-3    |                 | 108         | 96        | 126       | 127         |
| Marshfield-1 |                 | 107         | 98        | —         | 104         |
| Marshfield-2 |                 | 96          | 99        | 156       | 100         |
| Marshfield-3 |                 | 97          | 147       | —         | 98          |
| HW           | Arlington-1     | 120         | 140       | —         | 123         |
|              | Arlington-2     | 102         | 99        | —         | 100         |
|              | Ashland-1       | 111         | 104       | 114       | 120         |
|              | Ashland-2       | 115         | 107       | 103       | 117         |
|              | Marshfield-1    | 101         | 99        | 106       | 101         |
|              | Marshfield-2    | 104         | 113       | 100       | 105         |

† See Table 1 for definitions and characteristics.

‡ No harvest.

regardless of potential differences in spatial variation patterns across locations or years.

Nearest neighbor analysis across locations and years reduced the LSD for comparing entry means across



**Fig. 1. Relationship between relative efficiency (RE) of nearest neighbor analysis conducted on total forage yield or computed as the weighted average of the REs for individual harvests ( $y = -27.5 + 1.24x$ ,  $R^2 = 0.89$ ,  $P < 0.001$ ).**

locations and years for all methods and all of the cool-season grass trials (Table 4). The LSD values were reduced by 3 to 14%, depending on method and trial. The LSR values were reduced by 13 to 23% for the two orchardgrass trials, but 8% or less for the other trials. Values of LSR were not always reduced, because NNA sometimes had the effect of reducing the range among entry means, often a characteristic of analysis-of-covariance methods. The decreases in the LSD and LSR values for the two orchardgrass trials represent substantial improvements in precision, demonstrating an improved ability to detect differences among entry means.

**Table 3. Relative efficiency of nearest neighbor analysis compared with randomized complete block analysis for biomass yield of three switchgrass trials.**

| Experiment† | Location       | Year 1 | Year 2 | Year 3 |
|-------------|----------------|--------|--------|--------|
|             |                | %      |        |        |
| SW1         | Brookings, SD  | 126    | 107    | 116    |
|             | Arlington, WI  | 103    | 96     | 104    |
| SW2         | Mead, NE       | 97     | 96     | —      |
|             | Stillwater, OK | 118    | 103    | —      |
|             | Arlington, WI  | 100    | 101    | —      |
|             | Spooner, WI    | 93     | 91     | —      |
| SW3         | Arlington, WI  | 103    | 99     | —      |
|             | Marshfield, WI | 109    | 100    | —      |

† See Table 1 for definitions and characteristics.

**Table 4. Statistics for randomized complete block (RCB) analysis and three methods of spatial analysis combined across locations and years for six forage grass trials.†**

| Experiment‡ | Method§   | Mean  | Range               | LSD0.05 | LSR0.05 | RE  | $r_s$ |
|-------------|-----------|-------|---------------------|---------|---------|-----|-------|
|             |           |       | Mg ha <sup>-1</sup> |         | %       |     |       |
| OG1         | RCB       | 10.13 | 2.41                | 1.19    | 49.4    | —   | —     |
|             | Pretotal  | —     | 2.52                | 1.09    | 43.2    | 120 | 0.98  |
|             | Posttotal | —     | 2.61                | 1.21    | 43.0    | 113 | 0.98  |
|             | Pre-IH    | —     | 2.53                | 1.05    | 41.5    | 129 | 0.98  |
| OG2         | RCB       | 9.34  | 1.28                | 0.54    | 42.0    | —   | —     |
|             | Pretotal  | —     | 1.46                | 0.47    | 32.2    | 131 | 0.99  |
|             | Posttotal | —     | 1.34                | 0.50    | 36.1    | 124 | 0.99  |
|             | Pre-IH    | —     | 1.35                | 0.46    | 34.3    | 135 | 0.99  |
| SB1         | RCB       | 8.62  | 1.17                | 0.49    | 42.0    | —   | —     |
|             | Pretotal  | —     | 1.14                | 0.45    | 39.7    | 120 | 0.98  |
|             | Posttotal | —     | 1.18                | 0.46    | 39.3    | 119 | 0.99  |
|             | Pre-IH    | —     | 1.15                | 0.45    | 38.8    | 122 | 0.98  |
| SB2         | RCB       | 8.53  | 1.15                | 0.68    | 59.1    | —   | —     |
|             | Pretotal  | —     | 1.04                | 0.61    | 58.7    | 125 | 0.98  |
|             | Posttotal | —     | 1.11                | 0.63    | 56.5    | 117 | 0.99  |
|             | Pre-IH    | —     | 1.02                | 0.61    | 59.2    | 127 | 0.99  |
| SB3         | RCB       | 7.75  | 1.67                | 0.62    | 37.2    | —   | —     |
|             | Pretotal  | —     | 1.65                | 0.59    | 35.5    | 113 | 0.99  |
|             | Posttotal | —     | 1.65                | 0.60    | 36.4    | 109 | 0.99  |
|             | Pre-IH    | —     | 1.65                | 0.58    | 34.9    | 115 | 0.99  |
| HW          | RCB       | 7.82  | 0.70                | 0.50    | 72.5    | —   | —     |
|             | Pretotal  | —     | 0.68                | 0.49    | 71.9    | 107 | 0.99  |
|             | Posttotal | —     | 0.68                | 0.49    | 72.7    | 105 | 0.99  |
|             | Pre-IH    | —     | 0.68                | 0.48    | 71.3    | 109 | 0.99  |

† Range = maximum – minimum entry mean; LSR = least significant range = 100(LSD)/Range; RE = relative efficiency (relative to RCB design),  $r_s$  = rank correlation coefficient of entry means for each adjustment method with entry means for the RCB design.

‡ See Table 1 for definitions and characteristics.

§ Pretotal = preadjustment based on seasonal totals; Posttotal = postadjustment based on seasonal totals; Pre-IH = adjustment based on individual harvests.

Differences in relative efficiency, LSD values, and LSR values among trials probably do not reflect biological differences among species, such as tiller morphology, growth habit, and reproductive development. On an individual-harvest or individual-location-year basis, trial SB2 had the highest average relative efficiency (146%), followed by trials OG2 and SB3 (Table 2). Thus, the relative efficiency of the combined NNA across locations and years could not be predicted from the individual analyses. Similarly, a previous study showed no consistent differences in relative efficiency of NNA across a range of cool-season grass species, including both bunch grasses and sod formers (Casler, 1999b). Furthermore, relative efficiency of NNA was not related to block size (number of entries) in the current study or that of Casler (1999b).

There were relatively small differences in the relative efficiency of NNA for the three different methods of analysis (Table 4). Nevertheless, the preadjustment-by-harvest method (Pre-IH) always ranked highest in relative efficiency, with a 2 to 9% unit advantage over preadjustment on the basis of yearly totals (Pretotal). Postadjustment (Posttotal) always ranked last of the three methods. The average relative efficiencies of the three methods were 115% for Posttotal, 119% for Pretotal, and 123% for Pre-IH.

The relative advantage of the two preadjustment methods suggests a certain loss of information in the postadjustment method. Combining the NNA covariates across locations and years into two comprehensive covariates with only 2 df appears to dilute the advantages of NNA observed at individual location-years of a trial. Nearest neighbor analysis is an adaptation of analysis of covariance, in which the covariates are alternative forms of the dependent variable (yield). Each

covariate is a regressor variable, requiring fitting of a linear regression coefficient. The postadjustment method fits a single regression coefficient for each covariate, implicitly assuming constant slopes across locations and years. The assumption of constant slopes appears to be invalid for all six trials, as indicated by the inferior relative efficiencies for Posttotal. In contrast, the preadjustment methods fit potentially different slopes for each location-year combination (Pretotal) or each individual harvest (Pre-IH). This required more work and more df, but resulted in slightly greater improvements in precision.

The advantage of Pre-IH over Pretotal is likely because of the interaction of harvests with locations and years, which can be observed in Table 2. The analyses within locations and years established a certain degree of consistency and predictability between the individual-harvest analyses and the analysis of total yield within locations and years (Fig. 1). However, the relative adjustments made to each harvest were highly inconsistent across locations and years of a trial, sometimes greater for first, second, or third harvest, or sometimes near zero for all three harvests. These data suggest that the best-fitting NNA model would have a separate slope for each harvest-location-year, as was the case for Pre-IH.

These results raise the possibility that the optimal NNA model for trials such as these would be highly flexible, allowing for the possibilities that data from any individual harvest may or may not benefit from a NNA-type spatial analysis and that the adjustment slopes may differ from one harvest to another. Such a model would require a detailed analysis and decision-making process for the data of each individual harvest and relatively sophisticated program code for the combined model, building in options for zero adjustment or a flexible

**Table 5. Statistics for randomized complete block (RCB) analysis and nearest neighbor analysis (NNA) combined across locations and years for three switchgrass trials.<sup>†</sup>**

| Experiment <sup>‡</sup> | Method | Mean  | Range               | LSD0.05 | LSR0.05 | RE  | $r_s$ |
|-------------------------|--------|-------|---------------------|---------|---------|-----|-------|
|                         |        |       | Mg ha <sup>-1</sup> |         | %       |     |       |
| SW1                     | RCB    | 7.72  | 5.89                | 1.19    | 20.2    | —   | —     |
|                         | NNA    | —     | 6.12                | 1.15    | 18.8    | 108 | 0.99  |
| SW2                     | RCB    | 8.99  | 4.34                | 1.17    | 26.7    | —   | —     |
|                         | NNA    | —     | 4.30                | 1.13    | 26.3    | 106 | 0.99  |
| SW3                     | RCB    | 13.83 | 7.30                | 2.57    | 35.2    | —   | —     |
|                         | NNA    | —     | 7.08                | 2.54    | 35.7    | 102 | 1.00  |

<sup>†</sup> Range = Maximum – minimum entry mean, LSR = least significant range = 100(LSD)/Range, RE = relative efficiency (relative to RCB design),  $r_s$  = rank correlation coefficient of entry means for NNA with entry means for the RCB design.

<sup>‡</sup> See Table 1 for definitions and characteristics.

adjustment, varying by harvest, location, and year. It is our experience that such an exercise might be useful for some crop scientists and for a limited number of field trials, but is likely too complicated for routine cultivar testing.

While RCB designs are commonly employed in routine cultivar testing programs, our results and those of numerous other authors indicate that blocks may contain considerable internal variability. While such a phenomenon does not invalidate the use of a RCB design, it may significantly reduce the precision with which cultivars means are compared. The inconsistency in spatial adjustments across time, both within and among seasons, suggests that the spatial variability that remains within blocks of a RCB design may be transient in nature. This may arise from numerous biological and/or physical phenomena that interact to influence differences in soil characteristics among plots, such as plot-to-plot or treatment variability in forage yield, nutrient removal rates, root production, and tiller density. However, despite the transient nature of spatial variability in these trials, these effects were sufficiently consistent that they resulted in spatial variability that could be detected across harvests and years. While the postdictive use of NNA generally resulted in improved precision for comparing cultivar means, the generally transient nature of spatial variability and the inconsistency across species limits the reliable use of observed spatial variability patterns in laying out blocks for future blocking designs.

There were no significant changes in ranking of entry means for any trial or any method of adjustment (Table 4). These results suggested that the RCB design was sufficient to randomly distribute the entries with respect to spatial variation at each location. Low rank correlations would reflect a nonrandom distribution of entries with respect to spatial variation patterns, resulting in differential adjustments to entry means across entries. This did not occur in this study. Thus, NNA had the advantage in this study of improving precision for estimates of entry means, but not in improving the accuracy of the estimates. These high correlations also indicated that spatial adjustment method had no effect on the ranking of entries in these trials.

The potential increases in precision for cultivar means across locations and years via NNA will enable better detection of differences among cultivar means in multi-location trials. Genetic gains for forage yield in forage

crops are very small, often difficult to detect even after several years of selection and breeding (Casler, 1998). Poor precision due to spatial variation will reduce the ability to detect small changes in forage yield means. The NNAs proposed in this study appear to be helpful for improving the ability to detect small differences in forage yield means for multiple-harvest forage grass trials.

The combined analyses across locations and years for the three switchgrass trials showed a slight gain in relative efficiency for NNA (Table 5), similar to that observed for the analyses within locations and years. These spatial analyses had little effect on LSD or LSR values, further suggesting that there may be a photoperiod or buffering effect that homogenizes spatial variation for these single-harvest biomass trials.

## ACKNOWLEDGMENTS

K.F.S. would like to thank the Victorian Department of Natural Resources and Environment and the CRC for Molecular Plant Breeding for providing travel funds to facilitate this study. The authors would also like to thank all of those collaborators who provided data and the permission to use their data for this study: E.C. Brummer, Iowa State University; Y. Papadopolous, Agriculture and Agri-Food Canada, Charlottetown, PE; L.D. Hoffman, The Pennsylvania State University; A. Boe, South Dakota State University; C.M. Taliaferro, Oklahoma State University; and K.P. Vogel, USDA-ARS, Lincoln, NE.

## REFERENCES

- APPEC. 1996. APPEC; protocols and agreements (rules) for the conduct of merit testing trials of pastures. Australian Pasture Plants Evaluation Committee, Dep. of Natural Resources and Environment, Ballarat, VIC, Australia.
- Benedict, H.M. 1941. Effect of day length and temperature on the flowering and growth of four species of grasses. *J. Agric. Res.* (Washington, DC) 61:661–672.
- Brownie, C., D.T. Bowman, and J.W. Burton. 1993. Estimating spatial variation in analysis of data from yield trials: A comparison of methods. *Agron. J.* 85:1244–1253.
- Casler, M.D. 1998. Breeding annual and perennial cool-season grasses. p. 23–47. *In* J.H. Cherney and D.J.R. Cherney (ed.) *Grass for dairy cattle*. CABI Publ., Wallingford, UK.
- Casler, M.D. 1999a. Repeated measures vs. repeated plantings in perennial forage grass trials: An empirical analysis of precision and accuracy. *Euphytica* 105:33–42.
- Casler, M.D. 1999b. Spatial variation affects precision of perennial cool-season forage grass trials. *Agron. J.* 91:75–81.
- Casler, M.D., and D.J. Undersander. 2000. Forage yield precision, experimental design, and cultivar mean separation in alfalfa cultivar trials. *Agron. J.* 92:1064–1071.

- Casler, M.D., K.P. Vogel, J.A. Balasko, J.D. Berdahl, D.A. Miller, J.L. Hansen, and J.O. Fritz. 2000. Genetic progress from 50 years of smooth brome grass breeding. *Crop Sci.* 40:13–22.
- Cullis, B.R., and A.C. Gleeson. 1989. Efficiency of neighbour analysis for replicated field trials in Australia. *J. Agric. Sci. (Cambridge)* 113:233–239.
- Cullis, B.R., and A.C. Gleeson. 1991. Spatial analysis of field experiments—An extension to two dimensions. *Biometrics* 47:1449–1460.
- Cullis, B.R., B. Gogel, A. Verbyla, and R. Thompson. 1998. Spatial analysis of multi-environment early generation variety trials. *Biometrics* 54:1–18.
- Gleeson, A.C., and B.R. Cullis. 1987. Residual maximum likelihood (REML) estimation of a nearest neighbour model for field experiments. *Biometrics* 43:277–288.
- Kempton, R.A., J.C. Seraphin, and A.M. Sword. 1994. Statistical analysis of two-dimensional variation in variety yield trials. *J. Agric. Sci. (Cambridge)* 122:335–342.
- Lin, C.S., M.R. Binns, H.G. Voldeng, and R. Guillemette. 1993. Performance of randomized complete blocks in field experiments. *Agron. J.* 85:168–171.
- Littel, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS systems for mixed models. SAS Inst., Cary, NC.
- Smith, K.F., and G.A. Kearney. 2002. Improving the power of pasture cultivar trials to discriminate cultivars on the basis of differences in herbage yield. *Aust. J. Agric. Res.* 53:191–199.
- Van Wijk, A.J.P., and D. Reheul. 1991. Achievements in fodder crops breeding in maritime Europe. p. 13–18. *Proc. 16th Meeting of the Fodder Crops Section of Eucarpia*. Wageningen, the Netherlands. 18–22 Nov. 1990. Pudoc, Wageningen.